

The University of Chicago

PHYSIOLOGY OF PINES INFESTED
WITH BARK BEETLES

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1935

By
RALPH WILLIAM CAIRD

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PHYSIOLOGY OF PINES INFESTED WITH BARK BEETLES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 460

RALPH W. CAIRD

(WITH ELEVEN FIGURES)

Introduction

The great loss of timber in the southern states due to infestation by the southern pine beetle (*Dendroctonus frontalis* Zimm.) has necessitated the search for better means of control of this insect. This physiological study was made to obtain some clue as to the factors involved in the death of the trees. The situation is complicated by the penetration of the wood by various fungi which are always associated with the beetle attack (4, 5).

These studies are a part of the program conducted by the United States Bureau of Entomology on the Bent Creek Experimental Forest near Asheville, North Carolina. The work was done in the laboratories of the Appalachian Forest Experiment Station of the U.S. Forest Service under the direction of Dr. F. C. CRAIGHEAD. The scope of the work was greatly increased by the assistance given by Dr. W. C. BRAMBLE, of the Bureau of Plant Industry, and the loan of equipment by the botany department of the University of Chicago and the Bureau of Plant Industry.

Descriptive studies

METHODS FOR MOISTURE ANALYSIS

Dendroctonus frontalis is a primary bark beetle, capable of infesting healthy pines and maturing its brood. Severe outbreaks have been common in the Virginias and Carolinas, Tennessee, Georgia, Alabama, Mississippi, Louisiana, and Arkansas. It is especially destructive to *Pinus taeda*, *P. echinata*, and *P. virginiana* (6).

D. frontalis outbreaks usually center about a pine or group of pines which have been injured in some way by lightning, drought, girdling,

defoliation, fire, etc. To induce beetle attacks in the stand chosen for this study, cages of wire netting filled with bark containing beetles about to emerge were set up around the bases of scattered trees. As the beetles left the bark and attacked the inclosed portion of the tree, other beetles in the vicinity were attracted to the caged trees and the neighboring healthy ones. In this way it was possible to select uninfested trees and to keep a record of the dates of attack. Certain induced outbreaks furnished trees in the early stages of attack and others gave the later stages.

The studies reported in this paper were limited to a site of sandy clay soil about two acres in extent, which afforded a pure stand of *Pinus echinata* Mill. making rapid volume and height growth. Only dominant and co-dominant trees ranging in age from 20 to 25 years were used. These were 27 to 32 feet in height and were unbranched up to about 17 feet. Their diameter at breast height was from 4 to 6 inches and no heart wood had formed.

Many *D. frontalis* beetles simultaneously attack the tree at about mid-stem. The lower and upper portions of the trunk are usually infested a day or two later. Within three or four days after the mid-stem is infested, *Ips avulsus* Eich. is attracted to the injured tree and bores into the bark from the upper limit of the *D. frontalis* attack (20 feet) up to the leader. The two beetles cause similar injury, and in most cases are the only bark beetles involved. Usually the bark is perforated by the entrance holes from near the ground line to the leader. The holes bored through the bark are small. The size of *D. frontalis* is only about 3 mm. in length and 1 mm. in width; *I. avulsus* is somewhat smaller. The insects bore through the corky bark and tunnel in the phloem, but do not enter the wood at any time (fig. 1). The flow of resin into the wounds soon stops and the greater part of the galleries appear free from resin. At intervals along the tunnels ventilation holes to the outside are made. The entire life cycle of the beetles is completed in from 30 to 40 days, three to five broods developing in a season (6).

It was necessary to place the tree in dye solution to note which rings were carrying the transpiration stream. A method for suspending the tree in position in order to place the base in the solution was devised by Mr. HUCKENPAHLER. Two 2×4" timbers were nailed to

three trees in a line, one at 3 feet above the ground and the other at 12 feet (fig. 2). With the center tree supported in this manner, it was ready for the next step in the experiment. A section was removed from the tree by cutting the trunk through at 0.5 foot and again at 1.5 feet. After this section or disc was removed, a pail of



FIG. 1.—Beetle galleries and *Ceratostomella* (bluestain); tunneling in phloem by southern pine beetle and bluestain extending in vertical streaks from the galleries.

dye solution was inserted in the gap (fig. 2), adjusted so that the bottom of the suspended tree was immersed in the dye. A satisfactory dye solution contained 3 gm. of light green in 6 quarts of water.

Entrance of air into the tracheids was partially avoided by making the lower cut first and by replenishing the dye solution when necessary. The solution was often prevented from rising more than 3 or 4 feet the first day by the accumulation of resin on the cut surface. This difficulty was overcome by sawing off a disc about 1 inch

thick from the bottom of the suspended trunk each morning and evening for 3 days.

After the base of the trunk had been in the dye solution for 3 days (70 hours), the tree was taken down, limbed, and the trunk notched at 3-foot intervals in a straight line from the butt to the leader. A

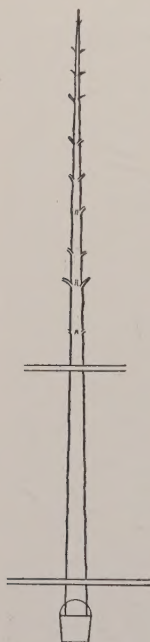


FIG. 2.—Method of suspending tree to place it in dye solution.

disc 2 inches thick for mapping and for moisture samples was removed at each notch. Diagrams showing the distribution of the dye and any discoloration due to *Ceratostomella* spp. were made by placing tracing paper over the face of the disc (fig. 3 A). The annual ring was used as the unit for sampling, rather than an arbitrarily measured depth. The tissues were grouped for moisture sampling as follows, beginning with the outside: phloem, first three rings (1-3 inclusive); second three rings (4-6); third three rings (7-9); any remaining rings in the center of the tree, called the "center" (fig. 3 B). The samples for moisture determination of the phloem were secured by removing the corky bark with a knife and stripping off the phloem. The wood for moisture determination was chipped off with a knife. The knife blade was placed next to the summerwood limiting the ring group (heavy black lines in fig. 3 B) and the wood chipped off by striking on the back of the knife. The fine chips lost water rapidly and it was found that the error involved was less if the weight of the sample was determined by weighing the disc before and after chipping rather

than by weighing the chips themselves.

The samples were dried in paper bags in a large Freas electric oven equipped with a fan and regulated to $100^{\circ}\text{C} \pm 1^{\circ}$. Heating was continued until none of nine heavy samples from different parts of the oven lost more than 0.04 gm. after 8 hours of continuous heating between weighings. The wet weight of the samples was usually about 35 gm. The average error due to various causes was probably well within 5 per cent of the true value.

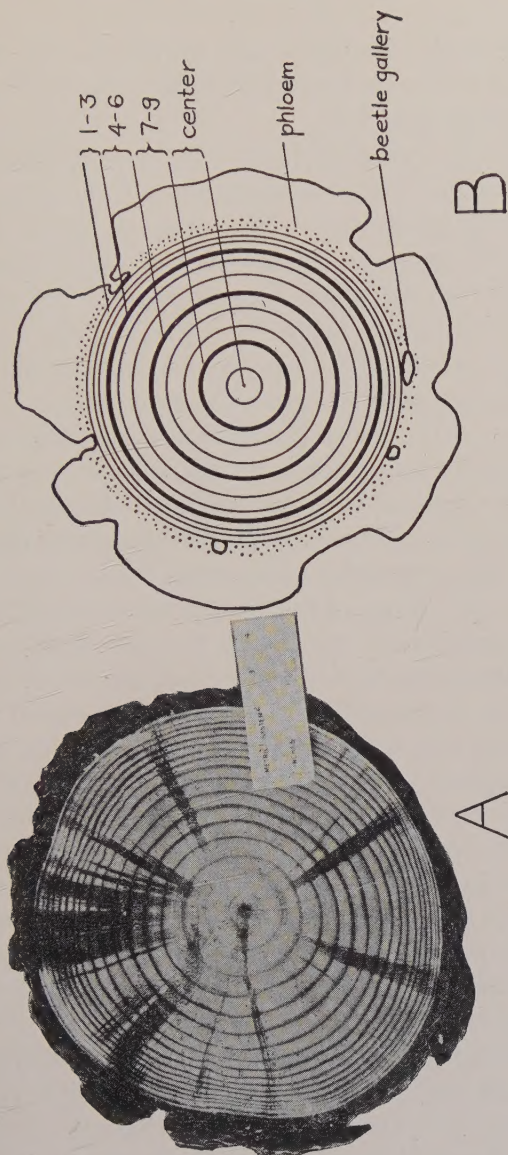


FIG. 3.—A, cross section of wood infested with *Dendroctonus frontalis* 30 days after attack (width of annual rings shown on rule); B, groups of rings taken to make composite samples for determination of moisture content.

THE HEALTHY TREE

The conduction of dye solutions and the distribution of moisture were determined for the healthy tree in order to form a basis of comparison with the diseased tree.

DISTRIBUTION OF DYE SOLUTIONS.—The general distribution of dye in a healthy tree is shown in figure 6 A. The main course of the dye solution is up the outer rings. It passes up the inner rings more slowly, and if the tree is allowed to remain in the solution all of the rings except the center ones become colored. Vertical conduction appears to be mainly up the springwood. The dye solution which first enters reaches only 3 or 4 feet during the first 24 hours, owing to the accumulation of resin on the cut surface. During the second 24 hours, after removing a section of the base to secure a clear surface, the dye may reach mid-stem or under some conditions the branches and leaves. Irregular one-sided distribution is often observed, although more frequently the distribution is uniform. The period of three days is probably sufficient for the dye to travel as far as it will in the outer layers of the healthy and diseased trees. Distribution may be irregular during the first two days, but on the third day the outer rings are uniformly dyed. As the top of the tree is approached the dye is confined to the outer one or two rings.

It is assumed in this study, without actual proof, that the lateral movement of the dye is the same as that of the original solution. The dye may diffuse into cells which are full of sap without any movement of the water which carried the dye up the trunk. The water undoubtedly advances up the stem more rapidly than does the dye, since the wood absorbs a certain amount of the latter.

MOISTURE GRADIENTS.—The moisture content of the various layers is not the same at each height up the tree, but presents definite trends or gradients from the base to the top, as indicated in figures 4 A and 6 A. An actual tree is represented, and although it is somewhat more regular than the usual healthy tree, it shows the general relationships very well. The outer three rings have the highest moisture content of the wood samples, the values averaging from about 110 per cent (dry weight basis) at the base to 175 per cent at the top. These higher values are probably due to the immature cells

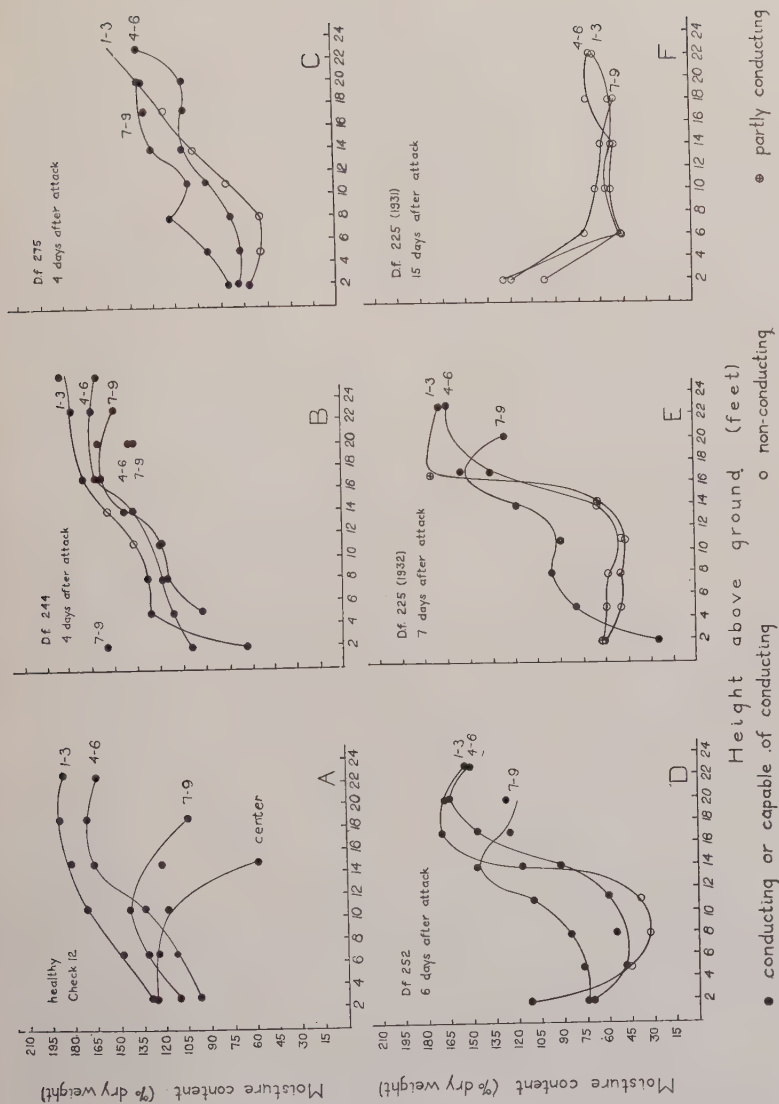


FIG. 4.—Successive stages in drying of tree and loss of ability to conduct dye solutions

adjoining the cambium and to the water of the transpiration stream. Rings 4-6 have a lower moisture percentage than rings 1-3, but have a similar type of curve. The remaining ring groups have intermediate values at the base which result in a crossing of the curves. In the top of the tree the values of all the groups are widely separated, a sharp drop in moisture content being frequently observed.

The reasons for the moisture gradients have not been investigated experimentally. The annual rings of these trees are narrow at the base of the tree and wide at the top, which probably results in denser wood at the base, leading to a lower moisture percentage. The sharp drop in the moisture content of a given group of rings when the top of the tree is approached may be the result of a functional change as these rings become the center of the tree. For example, at the base of the tree the fourth ring probably carries a portion of the transpiration stream, a function which it loses farther up the trunk. The rather slow passage of the dye solution up the center of the tree and its more rapid movement up the outer rings indicate the possibility of a gradual shift in the transpiration stream from many layers at the base to only one or two outside layers at the top of the tree.

The wide range of values obtained for the trees as indicated in figure 5 (*A*, *B*, *C*) is partly due to variations in height, diameter, and crown development, as well as to the introduction of a dye solution. Any dominant or co-dominant tree, picked at random from the stand, could be expected to have the typical graph form of the healthy tree, and to have the values fall within fairly definite limits. The diseased trees, as will be seen later, have a distinct graph form which varies significantly from that of the healthy trees. Sufficient work has been done with trees not steeped in solutions to indicate that the moisture relationships are general for the tree as it occurs in the field.

THE DISEASED TREE

The changes occurring when the beetles infest the healthy tree are marked. The outer rings fail to conduct dye solutions, drying takes place in these outer rings, and various fungi enter the wood.

DISTRIBUTION OF DYE SOLUTIONS.—No apparent difference from the healthy tree can be noted in the general distribution of dye in

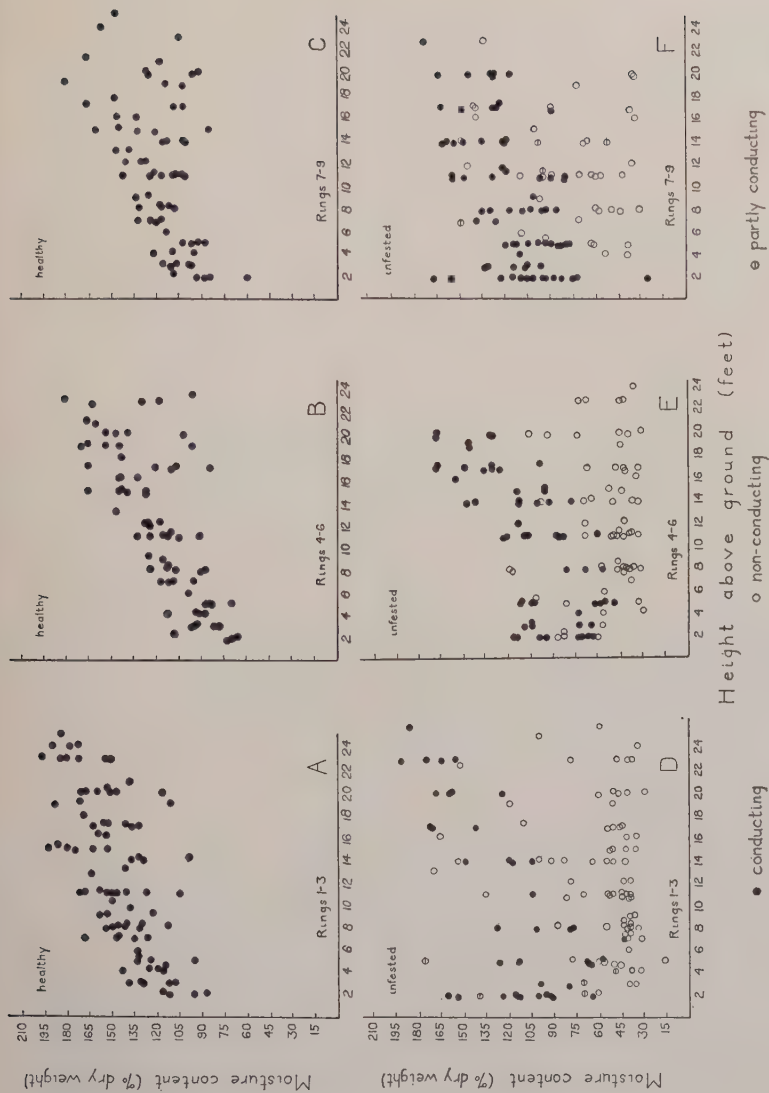


FIG. 5.—Moisture content of healthy trees as compared with trees infested with bark beetles. Percentage of moisture in wood of infested trees drops below that of healthy trees. Wood containing less than 75% moisture is incapable of conducting dye solutions.

the diseased tree during the first, second, or third days after the beetles' attack. The dye passes up to the leaves in the outer rings. Examination during the second and third days shows light colored patches in the wood along beetle galleries, caused by the accumulation of air in the cells as drying takes place. The first annual ring or two become non-conducting on about the fourth day after attack. As the outer rings become non-conducting, the transpiration stream is carried in rings which are closer and closer to the center of the tree (figs. 6, 8). In the early stages of attack the position of the dye in the top of the tree is the same as in the healthy tree. In later stages of the disease no dye reaches the top, owing to the stoppage of conduction at mid-stem.

DRYING OF WOOD.—The moisture content of the diseased tree soon falls below that of the healthy tree (fig. 4). The phloem becomes brown and commonly its moisture content falls from 225 to 100 per cent (dry weight basis). Rings 1-3 dry and become non-conducting before rings 4-6, and the latter before rings 7-9; that is, the drying progresses from the surface of the wood toward the center. Drying does not continue indefinitely but reaches a minimum moisture content at about 30 per cent. This lower limit is reached first by rings 1-3 at mid-stem. After 25 to 30 days the entire trunk is usually dry except for the base (figs. 4 *F*, 6 *F*). During subsequent decay the tree may become moist again, but such changes are outside this study.

Drying of the wood is correlated with loss of ability to conduct dye solutions, as is indicated in figures 4 and 5. As the wood dries it passes from a conducting to a non-conducting condition. The graphs, which contain all available data, serve to indicate a zone of demarcation lying between the wholly conducting and the wholly non-conducting values. This transition occurs in wood containing 65 to 85 per cent moisture (dry weight basis), as compared with the healthy tree values of from 100 to 185 per cent. This is a percentage figure, of course, and does not give the value of a single tracheid. The values presented are the sampling points in which the indicator dye was actually present in the wood.

FUNGAL PENETRATION.—The wounding of the tree by the beetles and the drying of the wood might be considered as the causes of the

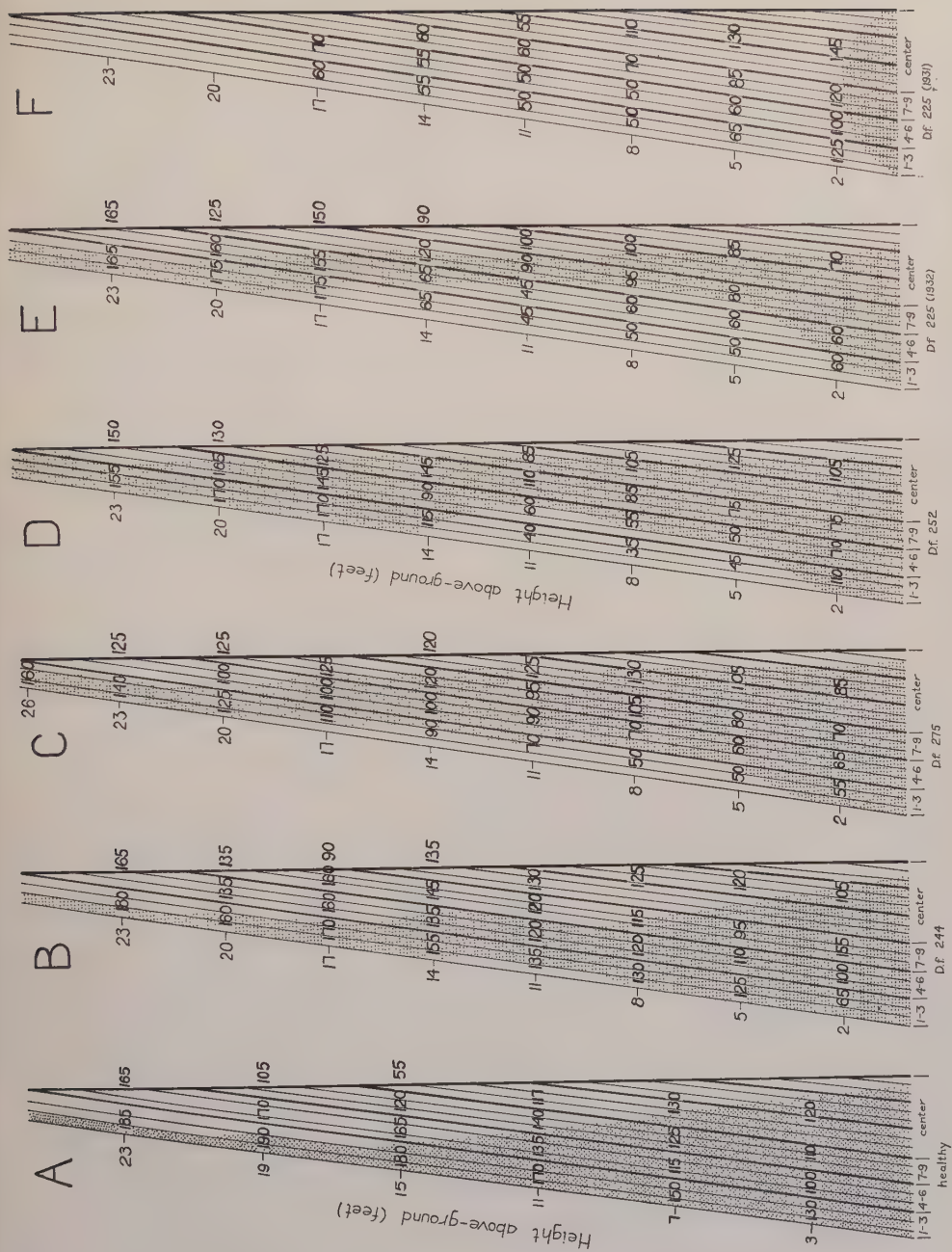


FIG. 6.—Loss of ability to conduct dye solutions as trunk dries. Moisture contents in heavy figures are from graphs in fig. 4

stoppage of conduction, but it will be seen from the following description of the penetration of fungi into the tree that the action of the fungi might in itself account for the death of the tree. Although it seems more probable that these various factors are interdependent, each must be examined as possible single causes. It was particularly desirable to determine what fungi penetrated the wood of the diseased tree and to establish the position of the fungi with reference to the ascending dye stream.

METHODS FOR FUNGAL ANALYSIS.—In preliminary work during the 1931 season thirteen trees attacked by beetles were sampled for fungi. The trees were sampled systematically at 3-foot intervals, 50 pieces of wood tissue, or plantings, being taken from each tree with about 700 plantings in all. Approximately 1000 cultures were handled. The sampling intervals chosen were too widely spaced and the data secured were insufficient for the purposes of the investigation. The work done during 1931 was improved upon in 1932 and the results are given in detail.

Trees representing progressive stages of the disease were placed in dye solution for three days before they were taken down and limbed. The bark was scored with a hand saw in a line from the base to the leader in order to orient the discs so that they might be arranged in the same position they occupied in the standing tree. The trunk was then cut into 1-inch cross sections which were numbered consecutively according to the inches above the ground. Enough discs were taken into the laboratory to satisfy the day's requirements and the remainder were stored in a refrigerator regulated to 5° C. Tests by Dr. BRAMBLE indicate that this temperature is sufficiently low to prevent appreciable growth of *Ceratostomella pini* Münch, molds, and bacteria and yeast, if they are not kept for more than five days. The length of time needed for sampling a tree was not more, and usually less, than three days.

Wood for fungal cultures was secured by cutting a block about 1 inch wide along a radius of the disc in such a way as to include all of the rings from the bark to the pith (fig. 7 A). After transferring the block to the inoculation chamber, the bark was removed and tissue taken from the springwood of each annual ring. The wood was split by placing a sterile scalpel along the springwood of the annual ring

which was to be sampled and striking down gently with another object. Care was taken to start the splitting with a sterile scalpel and then to pry with the scalpel to complete the splitting without touching the area from which the tissue was to be taken. A second sterile scalpel was used to cut off a thin slice of wood about 3×4 mm. in surface dimensions from the center of the exposed surface of the block. Five plantings each were taken from the first two rings, and

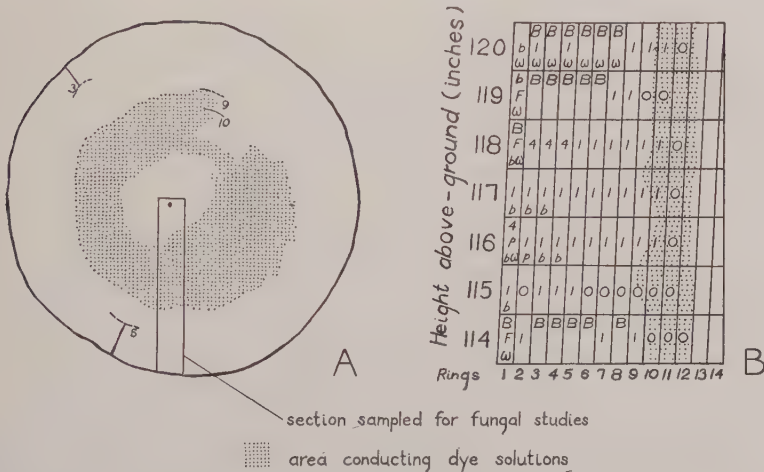


FIG. 7.—A, section of *D. frontalis* 355 at 120'' sampled for fungal studies. *Ceratostomella* apparent to the eye is indicated by heavy lines to the third and fifth rings. B, actual data collected for *D. frontalis* 355 at 114 to 120''. Key: B, *Ceratostomella* (blue-stain); 1, fungus no. 1; w, bluestain wedge observable in wood; O, negative cultures; b, bacteria and yeast.

placed on malt agar in petri dishes. One planting each was taken from the successive rings toward the center until a point two or three rings within the area dyed by the indicator solution was reached (fig. 7 B). The latter plantings were placed on malt agar slants in test tubes. The tissue was pressed into the agar so as to have a portion imbedded. The only culture medium used was malt agar, upon which most fungi which decay wood will show some growth. This was prepared from Bacto-malt-agar, according to the directions supplied by the Digestive Ferments Co. of Detroit, Michigan. Asheville city water was used. The pH of the medium was not adjusted and hence was approximately 5.5. A small amount of work was done

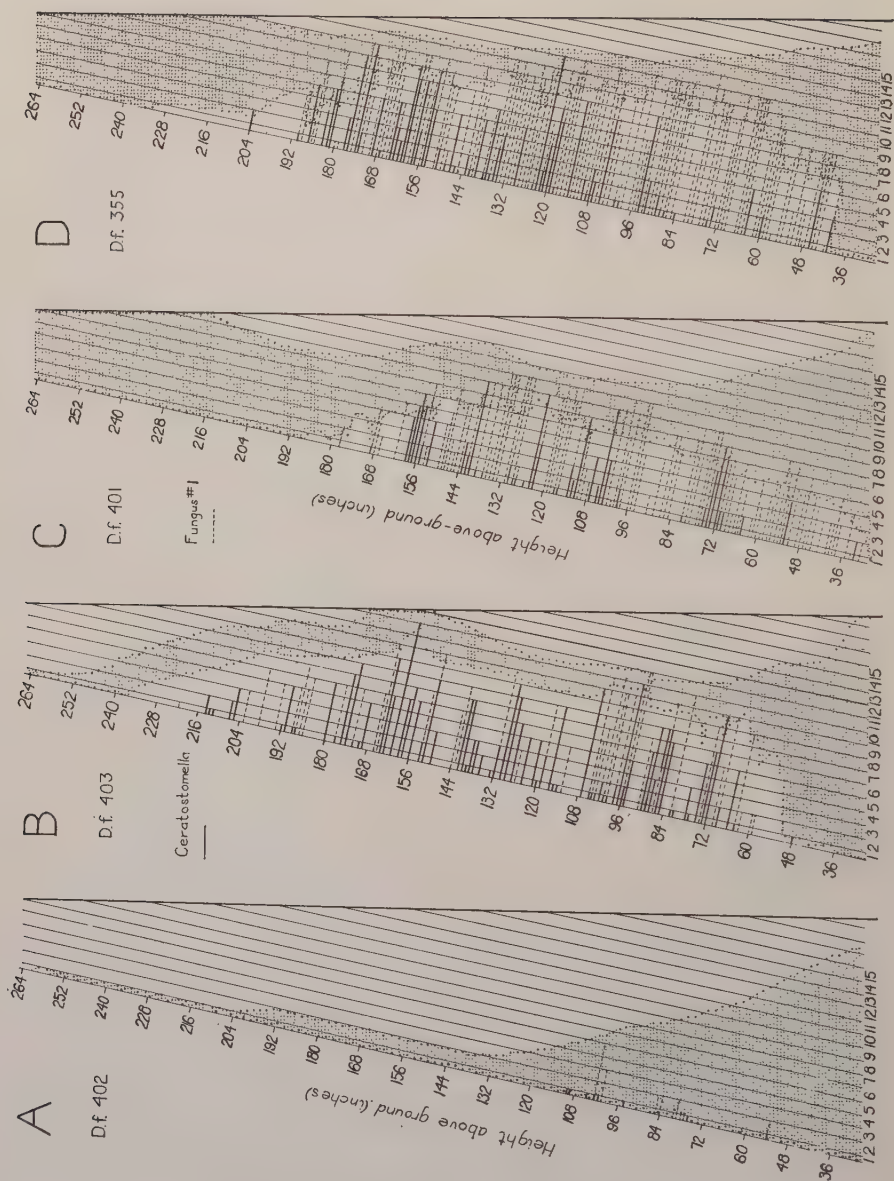


FIG. 8.—Penetration of *Ceratosomella* and fungus no. 1. Inclosed shaded area indicates wood conducting dye solutions. In *D. frontalis* 403 no samples were taken from 108 to 113", 144 to 149", or 180 to 186".

with malt agar prepared in the laboratory from a popular brand of malt syrup, using 45 gm. of syrup to 1000 cc. of water and 20 gm. of agar.

Since the data collected by these methods lose their significance if there is no assurance that the fungi isolated were actually growing in the wood and were not merely contaminations, precautions were taken to reduce the number of contaminations as much as possible. Inoculation chambers were used, the cultures were stored in large museum jars, and the mold fungi destroyed without opening the containers. The cultures were kept from 10 to 14 days, which was longer than necessary for the fungi to show distinctive colonies. Cultures designated as "sterile" at the end of this time remained so if kept for four weeks or longer. The fungi were recognized by their habits of growth on the malt agar. The great majority of the cultures appeared to be of single fungi, but no attempt was made to isolate them as such. The fungi easily recognized were *Ceratostomella* spp., *Trichoderma lignorum*, *Penicillium* sp., *Aspergillus* sp., and "bacteria and yeast." The growth habit of fungus no. 1 is distinctive although the lack of fruiting bodies has prevented its identification. No description of the growth habits of this fungus has been published.

The *Trichoderma* cultures were identified as *T. lignorum* by Dr. BRAMBLE and Dr. RUMBOLD. A culture from the isolation study designated *Ceratostomella pini* by Dr. BRAMBLE was confirmed by Dr. RUMBOLD of the Forest Products Laboratory.

The chief error in designation of the fungi was probably the failure to observe fungus no. 1 when it occurred with *Ceratostomella* (fig. 7 B). Bacteria and yeast were occasionally missed when growing with higher fungi, and it is possible that yeast with hyphae were classified with higher fungi. These errors are probably negligible when the number of cultures examined and the use to which the data are put are considered.

Figure 7 shows the detailed results upon which the description is based. Figure 7 A shows the irregular distribution of the dye and figure 7 B gives a portion of the actual notes. In making up the graphs (fig. 8) the following rules were adopted:

1. Where fungus no. 1 occurs with *Ceratostomella*, only *Ceratostomella* is shown.

2. Where fungus no. 1 or *Ceratostomella* spp. were not found in some of the rings, the fungus is shown as present in all rings to the innermost one in which it is found.

3. Where notes are lacking as to dye conduction, the points where the data are available are connected with a smooth line.

FUNGAL STUDIES IN 1932.—The results of the 1932 study are given according to individual trees which represent different stages of the disease.

Tree no. 402 (fig. 8 A) represents an early stage of attack of *D. frontalis* about three or four days after infestation. The beetles were still extending their galleries and laying eggs. These beetles which first attack the tree and make the galleries along which the eggs are laid are designated parent-adults. The tree although only lightly attacked would no doubt have died if left standing. The indicator dye appeared in the outer ring along the line chosen for analysis except at four places. *Ceratostomella* was cultured in the first ring although not apparent to the eye. Fungus no. 1 was cultured in the dyed area of the fifth ring at 102 inches and had apparently penetrated into the wood more rapidly than *Ceratostomella*. Bacteria and yeast, *Penicillium*, and *Trichoderma* were found in the first ring.

In tree no. 403 (fig. 8 B) parent-adults, tiny larvae, and eggs of *D. frontalis* were present at mid-stem and *Ips avulsus* had entered the bark from the height of 14 up to 20 feet. Close correlation between the position of the dye and the position of the fungi was not expected, and exact notes as to the position of the dye for every inch were not always noted. The dye area shows indentations associated with the presence or absence of *Ceratostomella* or fungus no. 1. This is found in 24 instances where exact notes are available. These are the only fungi present to any great extent in the inner rings of the wood. *Ceratostomella* was secured in the first ring from approximately 40 per cent of the sampling points. From 52 to 63 inches the tissue appears to be sterile for the most part but not dyed. This may be due to the heavy tunneling of the bark by the beetles and the attend-

ant drying rather than to the *Ceratostomella* and the fungus no. 1 at 64 and 65 inches. It may be that in this tree the drying of the wood has advanced faster than the fungi, and that the drying may be acting alone, apart from any action of the fungi.

Tree no. 401 (fig. 8 C) had parent-adults and eggs present at the base, one-fourth to one-half grown larvae at mid-stem, and parent-adults, eggs, and tiny larvae at the top. Plantings to the number of 2520 were taken from 25 to 185 inches. The same relation between conducting (dyed) areas and non-conducting areas appears to hold. This tree is remarkable for the small amount of *Ceratostomella* found in the inner rings. It was frequently noted as being observable on the surface of the outer rings but was actually cultured in only 37 out of 150 sampling points. Fungus no. 1 was commonly found two or three rings within the dyed area and *Ceratostomella* in only four cases. The localized effect of bluestain is again seen in this tree.

Tree no. 355 had the parent-adults which had infested the tree about 6-8 days previously. Tiny larvae were in the base and mid-stem; eggs at 15 feet and higher. *Ips avulsus* had not as yet infested the top. The fungal diagram is based upon 3120 plantings taken at 1-inch intervals along the trunk from 36 to 204 inches. As shown in figure 8 D, the dye solution was taken up in the inner rings. The outer non-conducting rings were dry and almost solidly occupied by fungi. Fungus no. 1 was cultured from the conducting and partially conducting wood in a number of instances, while *Ceratostomella* was obtained in only two cases. The small amounts encountered from 40 to 90 inches make it seem improbable that this fungus was the cause of the stoppage of conduction observed. This area was dry and fungus no. 1 was obtained from it.

The brood of tree no. 400 was in the pupal and new-adult condition, about 30 days after infestation. The tree represents a very advanced stage of the disease, in which the leaves were yellowing and conduction up the stem had completely ceased. *Ips calligraphus* pupae were in the base up to about 25 inches, with *D. frontalis* above. The tree was sampled only from 8 to 60 inches. The wood was heavily "blued" by *Ceratostomella*, and this fungus was cultured from almost every planting. *Trichoderma* was also present in almost every

planting and quickly overran the agar. Bacteria and yeast were found even in the center of the tree. Other fungi were also present, but no attempt was made to separate the fungi in the mixtures.

DISCUSSION AND SUMMARY OF DESCRIPTIVE STUDIES

It is obvious that an insufficient number of trees were analyzed to explore the conditions fully, although the main features are brought out.

1. *Ceratostomella* and fungus no. 1 are the only fungi which are present in considerable amounts in the interior of the diseased tree. From inoculation studies *C. pini* is known to be able to kill pines, but fungus no. 1 has not been tested in this respect.

2. The great development of *Ceratostomella* which appears as darkened wedges (fig. 3 A) does not show until late in the disease. *Ceratostomella* may penetrate the wood to the ninth or tenth rings and not be apparent to the eye.

3. *Trichoderma*, *Penicillium*, bacteria and yeast, and other fungi enter the inner wood after conduction up the trunk has been stopped. Some of the fungal forms encountered are no doubt the common fungi which rot fallen timber.

4. An unexpected, close relationship between the positions of the fungi and the outer edge of the conducting zone (dyed area) was found. A more exact study with refined technique is desirable, with careful microscopic examination of the tissue. The precise relationship between the conducting zone and the fungi cannot be drawn from this study, since the methods were not sufficiently refined and the probability of the growth of the fungi during analysis is present.

5. No moisture samples were taken of the trees examined for fungi in 1932, but the later moisture studies indicate that non-conducting zones are drier than conducting zones, and that even the outer edge of the conducting zone has lost moisture. The loss of moisture and the positions of the fungi are intimately connected; this study, of course, is merely descriptive and gives no method for establishing causal relationships. It is pointed out, however, that drying of the wood may occur without the immediate presence of any fungus, and that in this sense drying is a constant accompaniment of the stoppage of conduction but the presence (action) of fungi is not. *Cera-*

tostomella appears to have mainly a localized effect. It seems probable that the fungi act in some way to accelerate the rate at which the tree dries, and thus produce conditions favoring their growth.

6. The position of the fungi might be purely incidental to the stoppage of conduction, but from the inoculation studies it seems probable that the fungi are actually concerned in the death of the trees.

Experimental evidence and general discussion

There is obviously need for experimental work to determine the action of the various factors involved when acting alone. This work is given briefly in this section, together with a discussion of the possible rôles of the various factors.

BEETLE GALLERIES

The entrances to the beetle galleries and the extensive tunneling of the phloem serve to expose the surface of the wood to the outer atmosphere. Drying could not occur if the bark were not opened by the beetles. This is substantiated by an experiment in which the entire bole of a pine infested with *D. frontalis* was given a heavy coating of grafting wax applied after the bark had been smoothed. New ventilation holes made by the beetles were promptly filled. The trees used were comparable intermediates about 30 feet tall and 4 inches in diameter at breast height. The moisture relationships after 33 days are shown in figure 9 A. The beetle brood failed to develop, *Ceratostomella* did not penetrate, so far as could be observed, and the leaves remained green. In a repetition of the experiment in 1932 the trunks of the waxed trees dried out, but in each case beetles made numerous ventilation holes through the wax.

The water which is lost as the trunk dries may pass out through the leaves or through the beetle gallery entrances, or both. That at least some of the moisture may leave through the entrances was indicated by a further experiment in which the trees were topped. If the limbs are removed from a beetle-attacked tree the trunk dries out fairly rapidly even though the foliage has been removed. Three pitch pines (*P. rigida* Mill.) were used in the study, the results of which are given in figure 9 B. Two of the trees had been infested by *D. frontalis* for about three days and one was a healthy tree of com-

parable size. One of the infested trees was limbed and the other was not. The limbed tree lost water from the trunk as indicated by the moisture condition after 33 days. Some water may have been lost through the cut ends of the branches but on the repetition of the experiment in 1932, trees in which the ends were covered with grafting wax showed the same general relationships as those not covered. The drying of topped trees infested with beetles has been described by NELSON in the 1929 cooperative experiment (2).

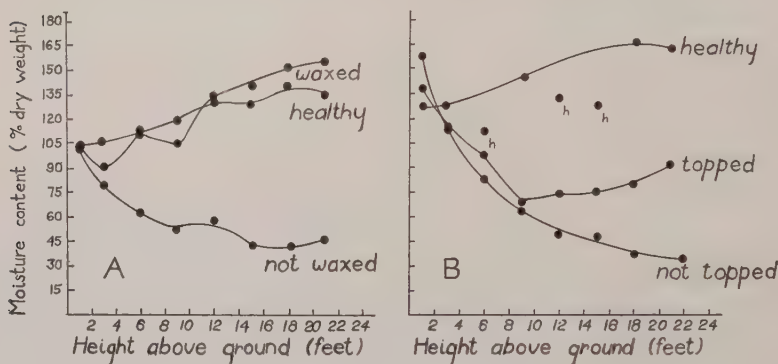


FIG. 9.—A, waxed-bole experiment: Tree infested with *D. frontalis* when waxed does not dry out to extent of a tree not waxed. B, topped tree experiment: Removal of limbs from tree infested with *D. frontalis* does not prevent drying out of trunk.

NATURAL DRYING

If the bark is stripped from a healthy pine for a distance of even 10 feet up the trunk, the tree does not die until long after the tree attacked by the beetles. However, the outer cells of the wood become impregnated with resin. If the surface of the wood is kept relatively dry and free of liquid resin by rubbing it with powdered resin, the outer wood dries and becomes non-conducting for the outer four or five rings, but only after a long period of time. It is possible that if the experiment were repeated and a resin-free surface maintained, more rapid drying and death of the tree due to simple drying might result and the effect of drying without the action of fungi might be determined.

INOCULATIONS

The rôle of *Ceratostomella pini* and probably of fungus no. 1 and the other fungi appears to be the speeding up of the drying of the bole of the tree. There is a close relation between the position the fungi occupy in the tree and the drying of the wood. This physiological study was limited to the broader aspects of the disease, since the investigation of the effect of the fungi on the cells requires intensive study. From the knowledge that *C. pini* is limited largely to ray parenchyma (3), it is possible to visualize how the action of a fungus in penetrating cell walls and killing the living ray parenchyma might make easier the loss of water and the entrance of air into the tissue penetrated and into vertical elements. Throughout this study, the gases present in the cells in the dried wood are referred to as "air," without actual proof that this is true. This assumption is made from the observation of the gradual drying from the outside toward the center, and because there is no apparent suction or pressure in the dry wood on exposing dried trees to dye solutions.

When the tree is inoculated with *Ceratostomella pini* there is drying and attendant passage of the dye solution around the diseased zone which is strikingly similar to the behavior of the dye solution in the trees attacked by the beetle. This has been described by NELSON (2). HUCKENPAHLER and WYGANT inoculated four trees with pure cultures of *C. pini* and analyzed them to determine what changes had occurred with regard to dye conduction and moisture content. After 25 days the effect of a 3 inch band of inoculum (fig. 10 A) about the tree at 11 feet is as shown in the graphs of figure 11. Check trees, with comparable injury but without the fungus, were not available at the time, but later experiments showed that trees inoculated with *Trichoderma lignorum* and with sterile rice did not suffer a loss of moisture in the wood. A tree inoculated with *C. pini* and left standing had not died after one year, although it seems probable that the trees analyzed for moisture and fungi would have died. The *C. pini* was recovered from the wood by plantings but was not redetermined by an expert. Detailed moisture determinations were made of four trees in which the sharp drop in moisture content shown in figure 11 is repeated. The same type of drying occurs in trees in-

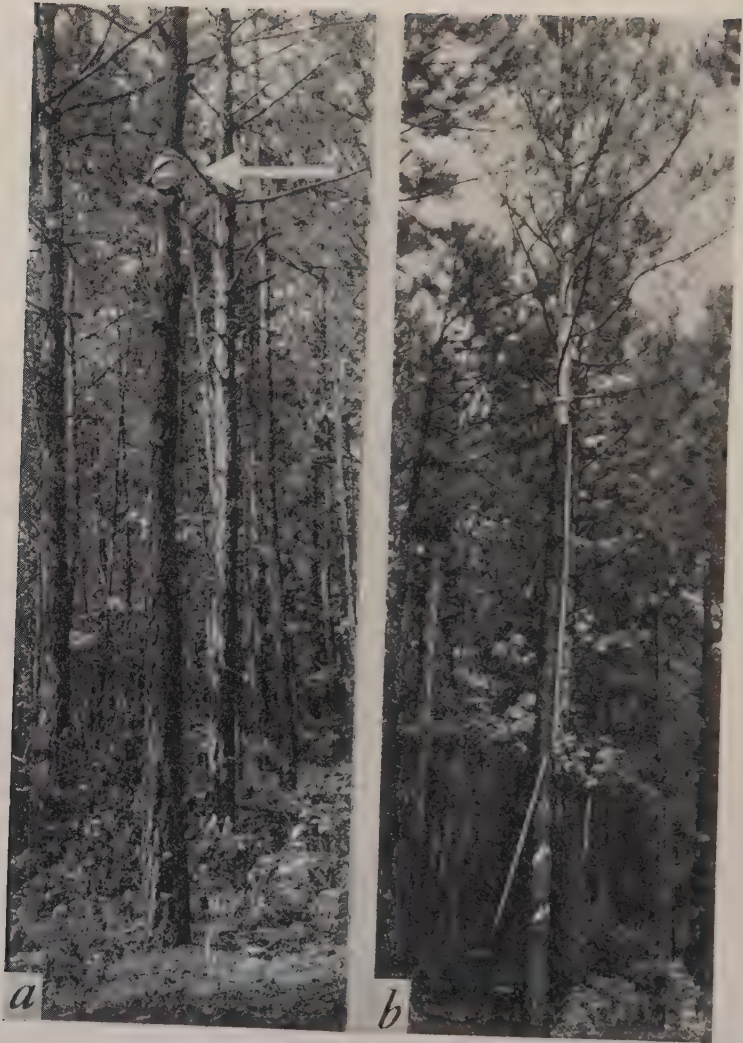


FIG. 10.—*A*, tree inoculated with *C. pini* in a 3 inch band at 12 feet. Inoculum applied as a poultice directly to wood by removing the bark which was then tacked back in place and the wound covered with grafting wax and waterproof cloth in an effort to prevent drying. *B*, tree killed by *C. pini* applied in a spiral band inoculation; tree protected from beetle attacks by wire screening and cloth.

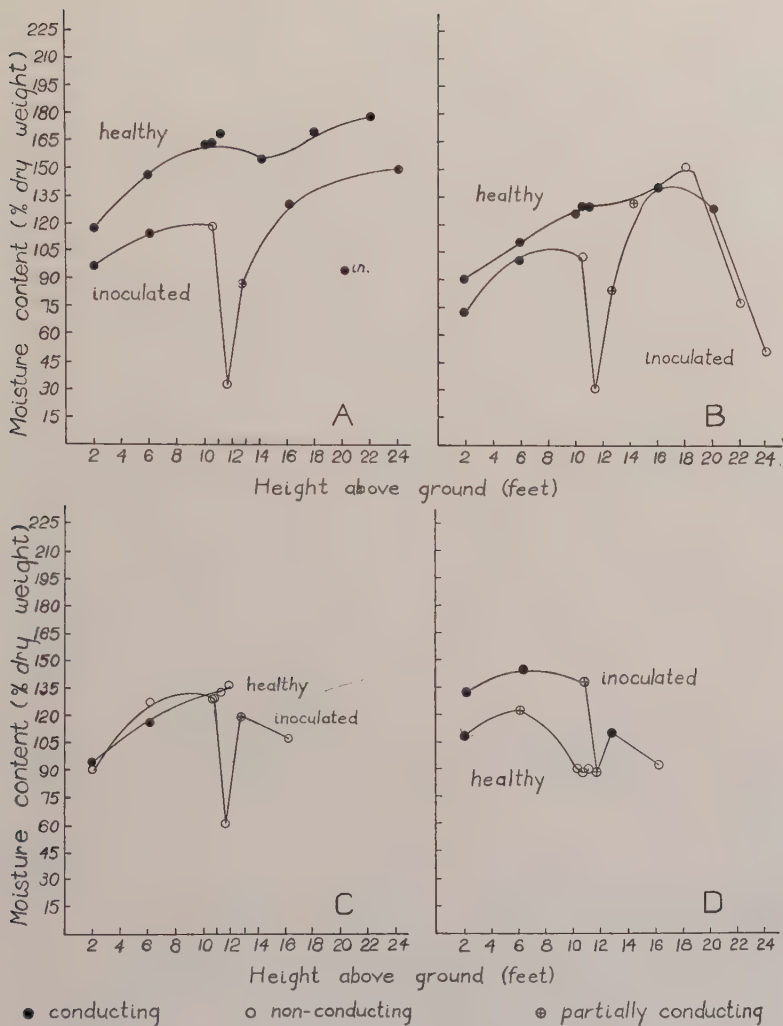


FIG. 11.—Inoculated tree no. 201 showing drying of wood at an inoculation band and loss of ability to conduct dye solutions. *C. pini* was recovered from the dried zone. A, rings 1-3; B, 4-6; C, 7-9; D, "center."

oculated with *Ceratostomella* in a spiral band (fig. 10 B), and in four bands at intervals of 2 feet. Later attempts to repeat the single band inoculations failed. The study of the tree infested with beetles, taken with the inoculation studies, serves to indicate that the death of the tree is due to the same cause in both instances. Future work might well make the inoculations a major study, since the factors can be isolated more easily.

Summary

1. *Dendroctonus frontalis* and *Ips avulsus* bore through the bark and tunnel in the phloem. The beetle inoculates the wood with fungi and the entrance holes and beetle galleries serve to expose the wood to the air.

2. The outer rings of the tree lose the capacity to conduct dye solutions successively from the first ring toward the center. This gradually advances to the center of the tree and cuts off the supply of water to the leaves which empty the top of water and die.

3. Failure of the outer rings to conduct dye solutions is intimately associated with drying of the wood. The suggestion is made that the accumulation of air in the rings, which accompanies the loss of moisture, forms a resistance sufficient to stop the vertical and lateral transport of water.

4. The failure of the wood to conduct dye solutions is also closely associated with the penetration of the wood by fungi. *Ceratostomella pini* and fungus no. 1 are practically the only fungi which penetrate deeply into the wood during the early stages of attack. Inoculations show that *C. pini* growing alone is able to kill the trees, but fungus no. 1 has not been tested.

5. From the close connection between the drying and the position of the fungi in the wood, it is suggested that the drying is accelerated by the action of the fungi on the cells.

6. The stoppage of conduction is probably also associated with the aspiration of the tori, as indicated by NELSON's work. He suggests that blocking of the pits of the tracheids by the tori might account for the stoppage of conduction.

I have received suggestions from many with whom I have discussed the problem. Mr. R. A. ST. GEORGE and Drs. R. M. NELSON

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